

# Mechanism of Microbial Metal Reduction

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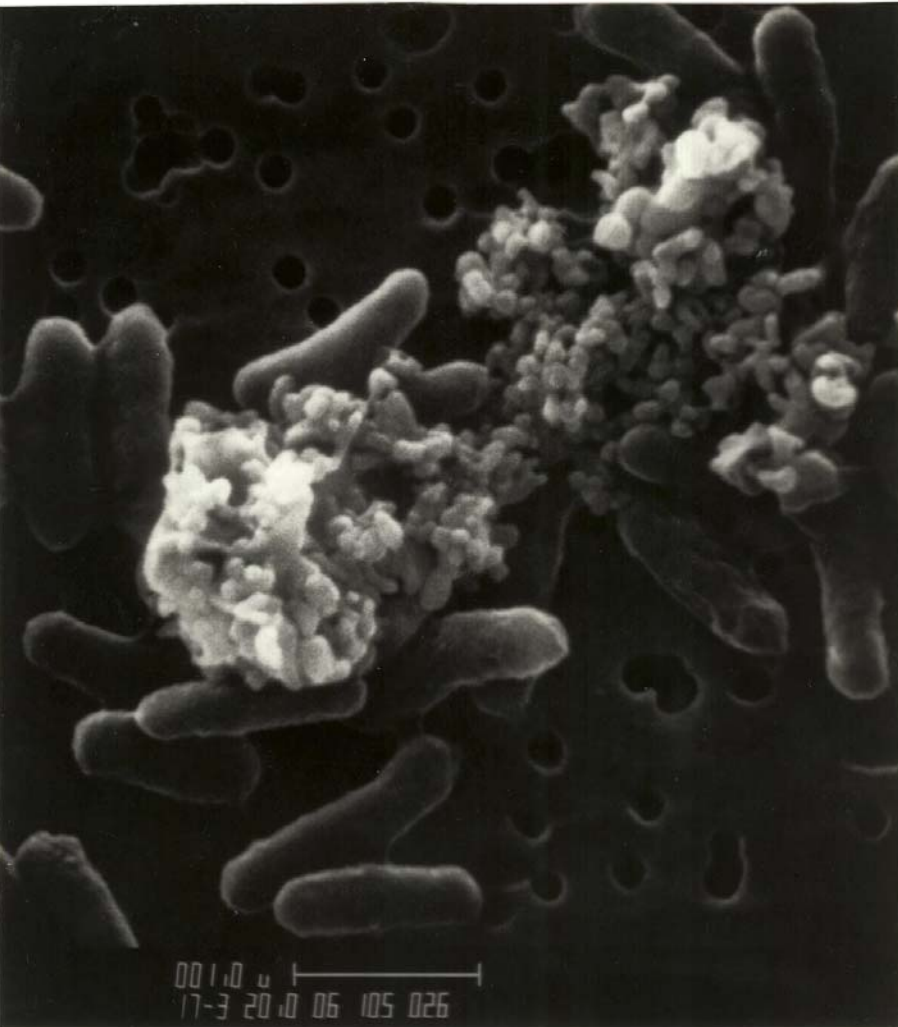
**DOE Biogeochemistry Grand Challenge with PNNL:**

**Fe: David Bates, Justin Burns, Christine Fennessey**  
**U: Jason Dale**  
**Tc: Amanda Payne**

**ERSP collaboration with Andy Neal at SREL:**

**Cryo HR-SEM and laser doppler velocimetry**

# Physiological problem associated with respiration on solid electron acceptors



## Potential solutions:

1. Secrete metal reductase out to OM
2. Solubilize and transport metal in to IM
3. Exogenous electron shuttles
4. Endogenous electron shuttles

Type I



Hemolysin by *E. coli*  
AQDS by MR-1

Type II



Cholera toxin by *V. cholera*  
Fe(III) reductase by MR-1?

Type V



Adhesins in *Bordetella*  
Autotransporter in MR-1

Type IV

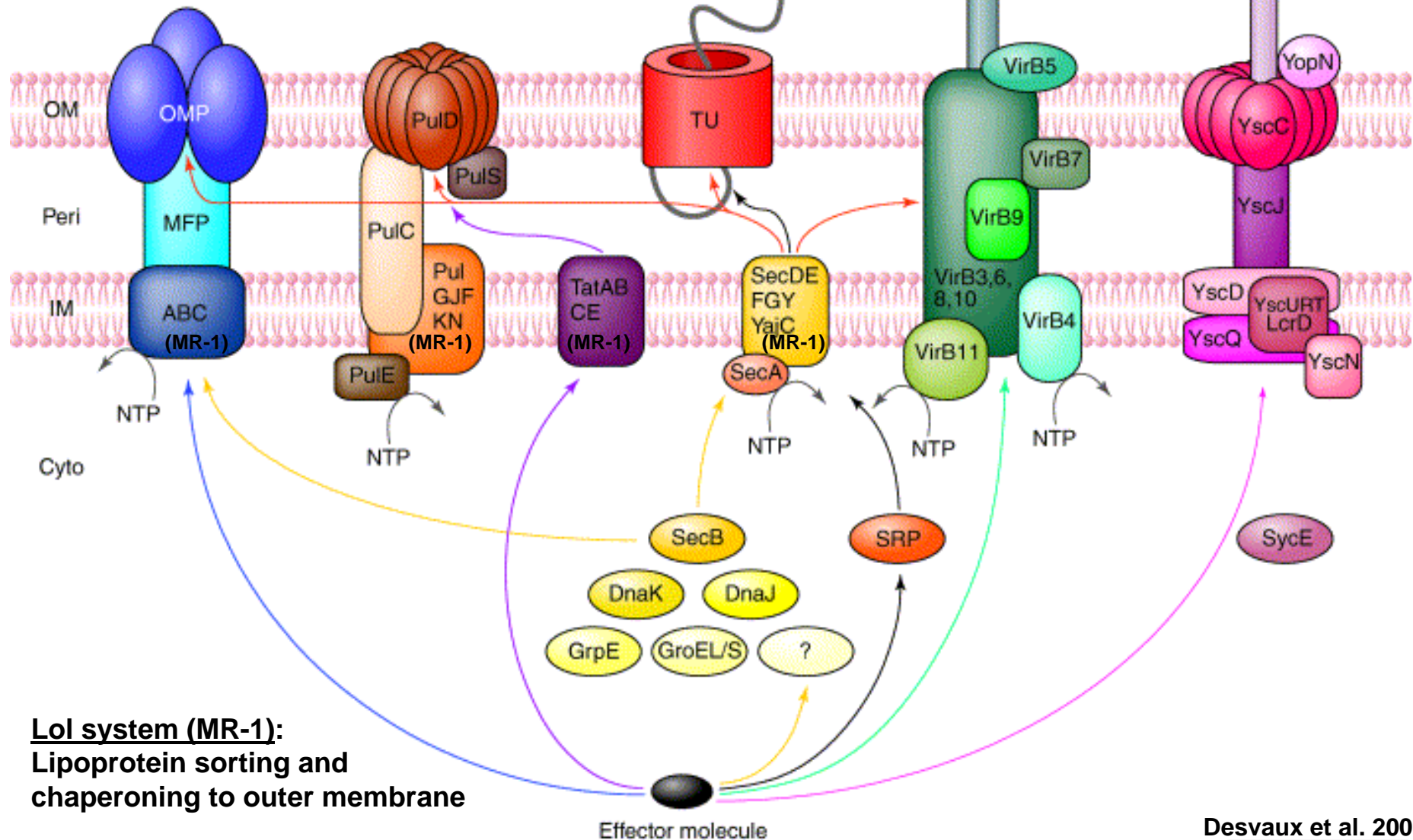


T-DNA in *A. tumefaciens*  
Not found in MR-1

Type III



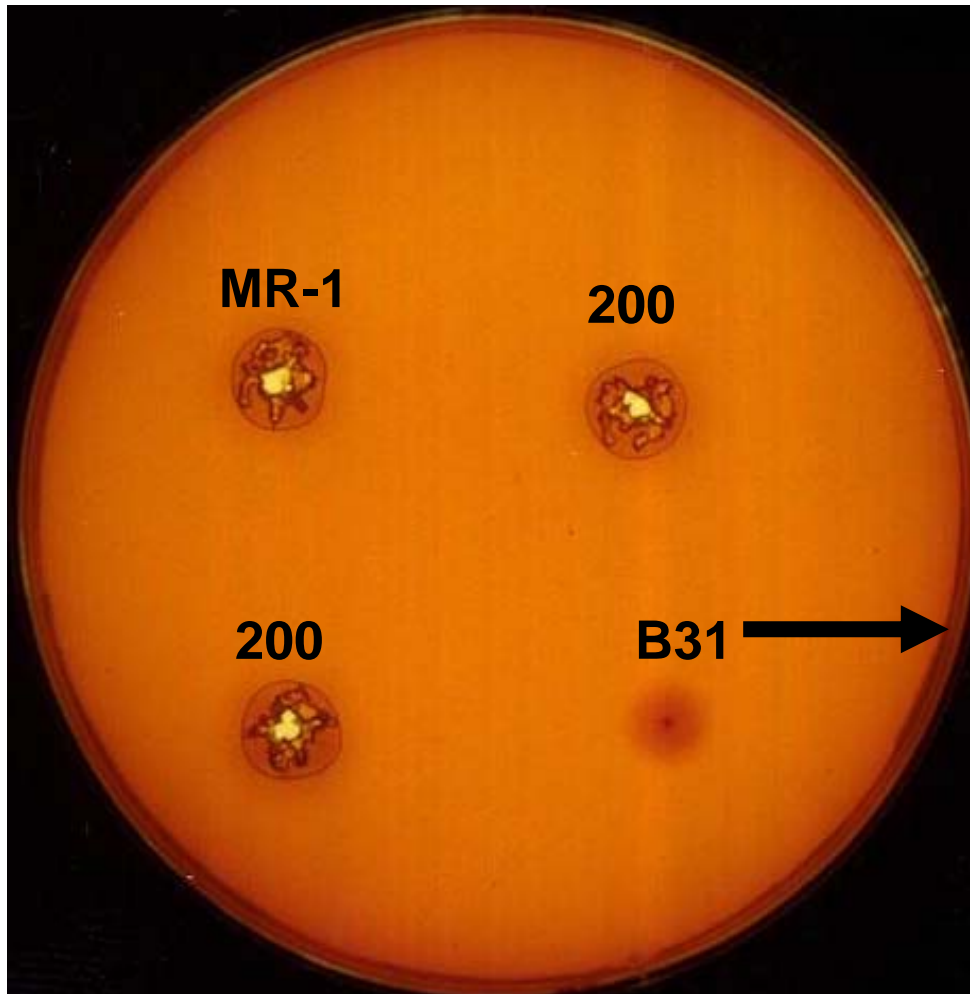
Euk Tx factors in *Yersinia*  
Not found in MR-1



Desvaux et al. 2004

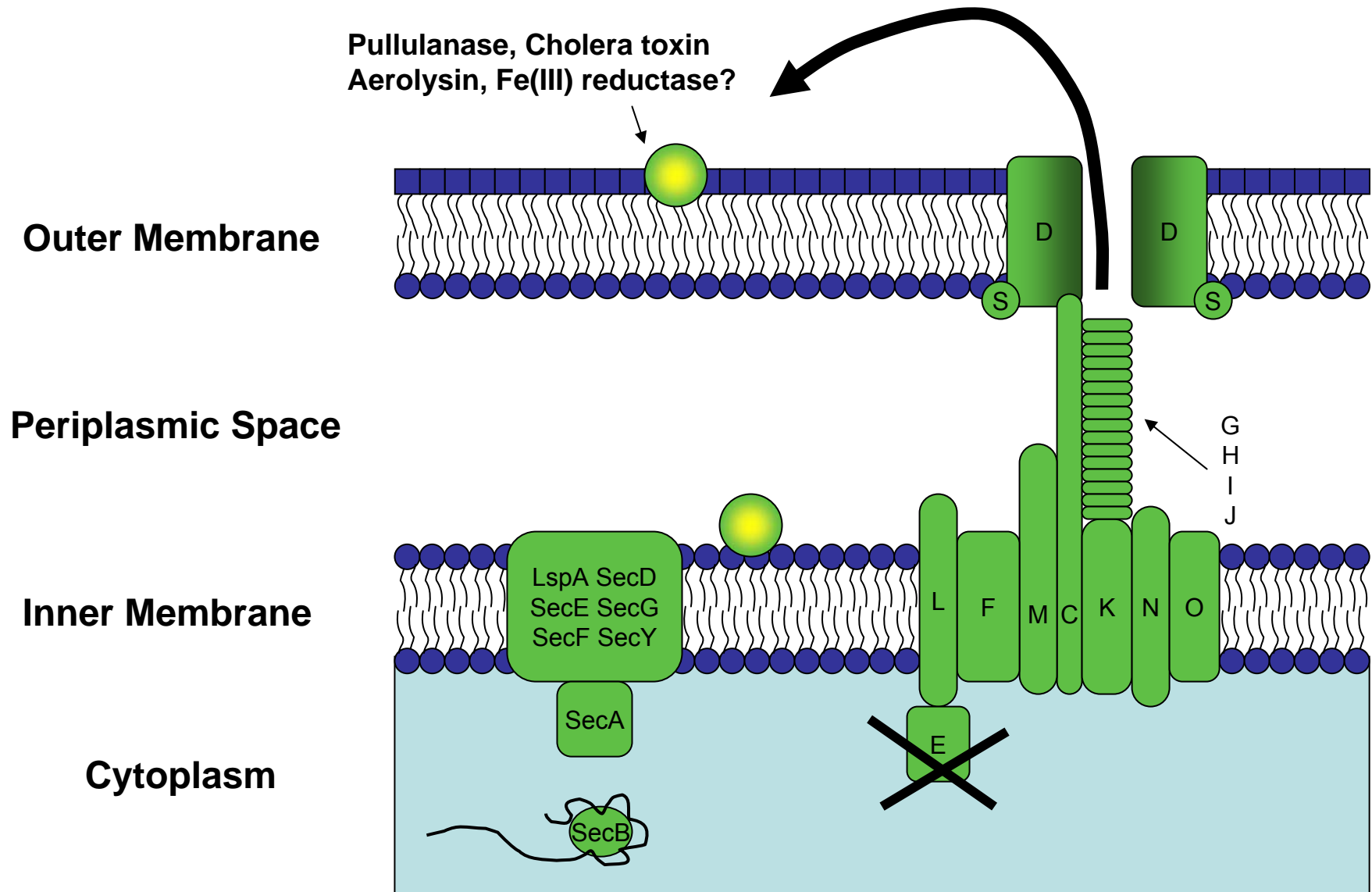
TRENDS in Microbiology

# AGR mutant phenotype - Fenton reaction

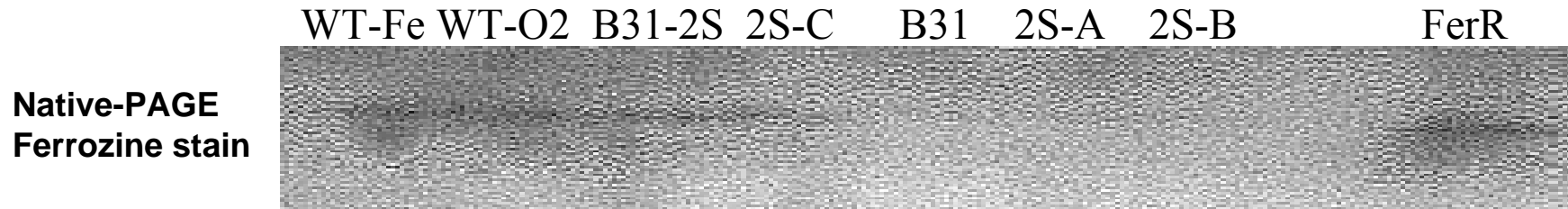


- 15,000 mutant colonies screened
  - Identified 72 that were Fe(III) respiration-deficient
  - 57 multiple deficiencies, 15 unique deficiency
- Does not respire solid Fe(III) or solid Mn(III/IV), yet respire 15 other electron acceptors [including soluble Fe(III) and soluble Mn(III)]**

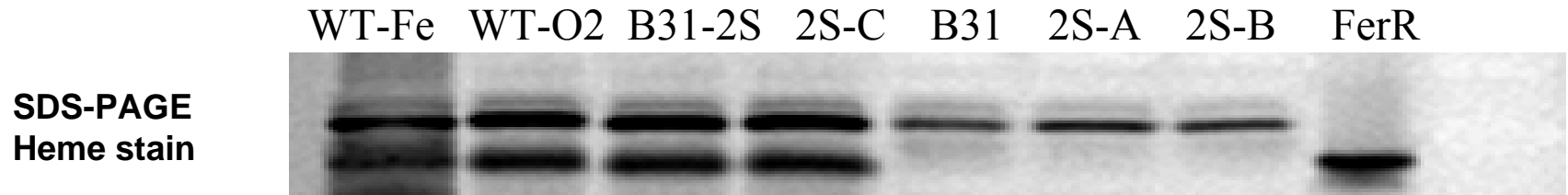
# B31 contains a mutation in GspE, the traffic ATPase of Type II protein secretion



## KCl wash to detach peripheral proteins from cell surface of WT and *gspE*



- Fe(III) reductase is missing from periphery of *gspE* mutant B31



## MALDI-TOF MS/MS analysis of excised 91 kDa heme-positive protein:

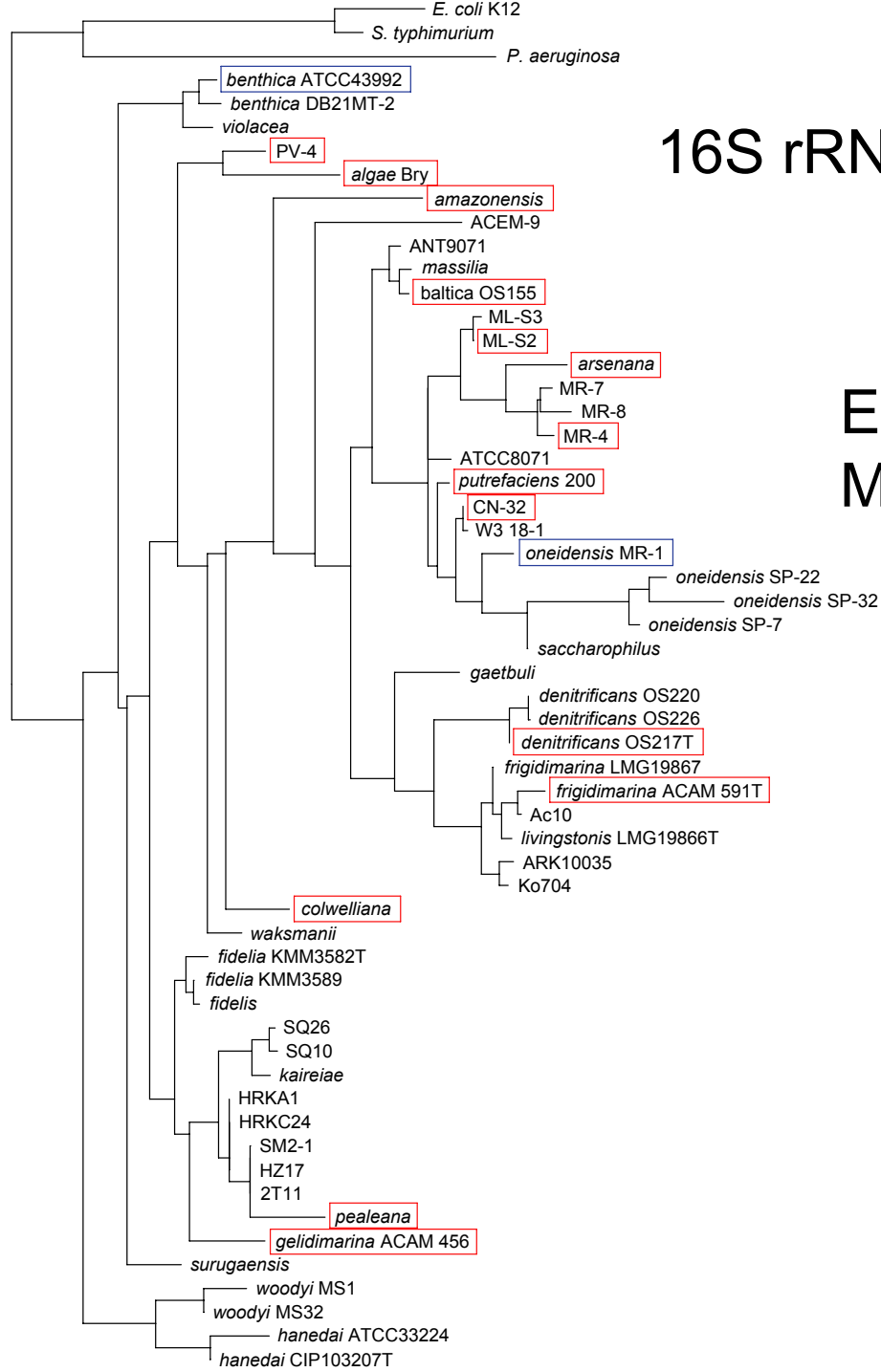
QYLIDETSDLTPAVK

- until January 2006:

“15 amino acid sequence obtained from MALDI MS/MS did not display homology to any predicted protein-encoding ORF in the sequence databases, including the *S. oneidensis* MR-1 genome”

## February 2006: MALDI-TOF MS/MS Identification of the c-type cytochrome missing from B31 outer membrane

Protein	Species	sequence homology to: QYLIDETSDLTPAVK	% Identity	% Similarity	e-value
MtrC	<i>Shewanella</i> sp. W3-18-1	QYLIDETCDLTPAVK (14/15)	100%	100%	0.0
MtrC	<i>S. putrefaciens</i> CN-32	QYLIDETCDLTPAVK (14/15)	78%	86%	0.0
MtrC	<i>Shewanella baltica</i> OS155	QYFGDETCDDVAAT- (7/15)	77%	84%	0.0
MtrC	<i>Shewanella</i> sp. ANA-3	QYFGDETCDDVAST-- (7/15)	75%	83%	0.0
MtrC	<i>Shewanella</i> sp. MR-7	QYFGDETCDDVAST-- (7/15)	75%	83%	0.0
MtrC	<i>Shewanella amazonensis</i>	EYLLDETCDDLPA-- (9/15)	65%	78%	0.0
MtrC	<i>Shewanella</i> sp. MR-4	QGL----- (2/15)	60%	71%	0.0
MtrC	<i>Shewanella frigidimarina</i> - 1	QFIGSEKA----- (2/15)	51%	64%	0.0
MtrC	<i>Shewanella frigidimarina</i> - 2	QFIGSEKA----- (2/15)	51%	65%	0.0
MtrC	<i>Shewanella</i> sp. PV-4	QYIGSQKA----- (2/15)	45%	59%	2e-151
MtrC	<i>Shewanella oneidensis</i> MR-1	QGL----- (2/15)	48%	59%	9e-141
MtrF	<i>Shewanella oneidensis</i> MR-1	QHFTSETC-----AA (4/15)	29%	48%	2e-71
MtrF	<i>Shewanella</i> sp. PV-4	QYFTSESCN----D (4/15)	30%	44%	4e-70
MtrF	<i>Shewanella</i> sp. ANA-3	QHFTSETC-----AA (4/15)	28%	45%	3e-69
MtrF	<i>Shewanella</i> sp. MR-7	QHFTSETC-----AA (4/15)	28%	44%	7e-69
MtrF	<i>Shewanella</i> sp. MR-4	QHFTSETC-----AA (4/15)	28%	45%	4e-68
MtrF	<i>Shewanella amazonensis</i>	QYFGSENCAT--- (5/15)	30%	44%	2e-65



# 16S rRNA tree - *Shewanella* genus

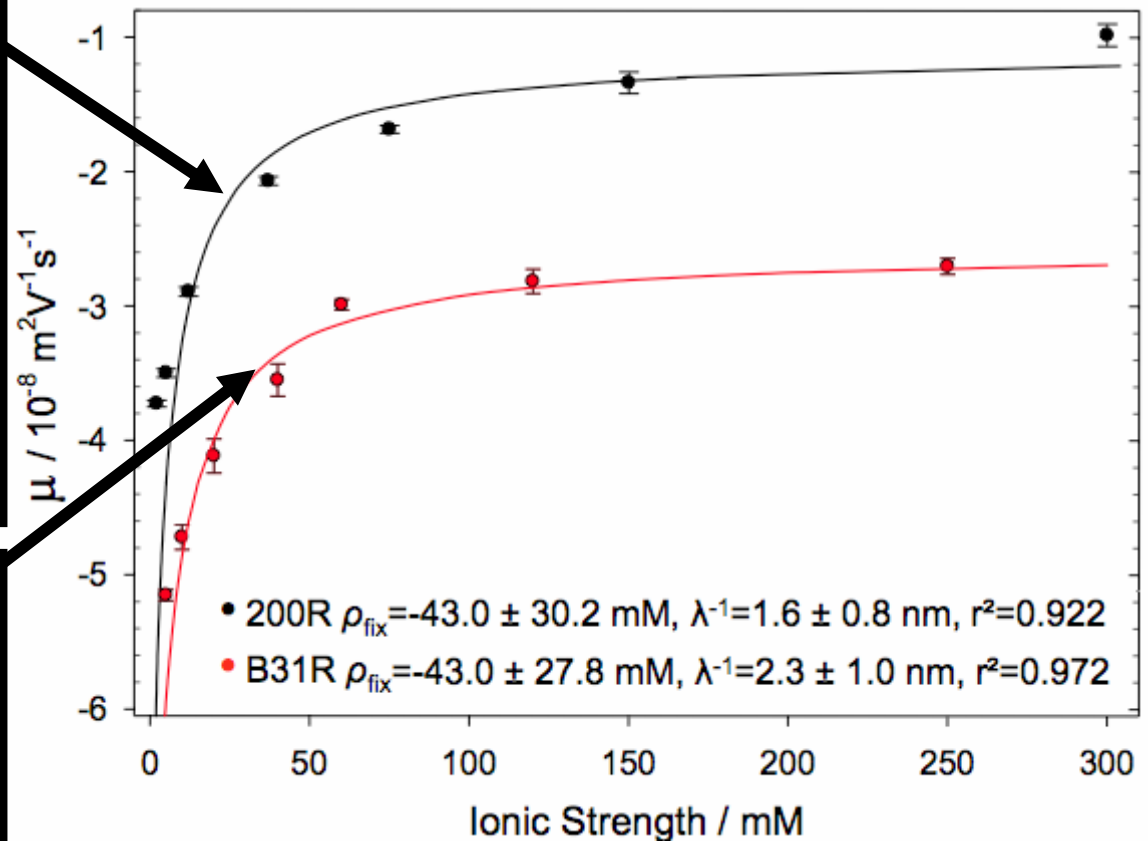
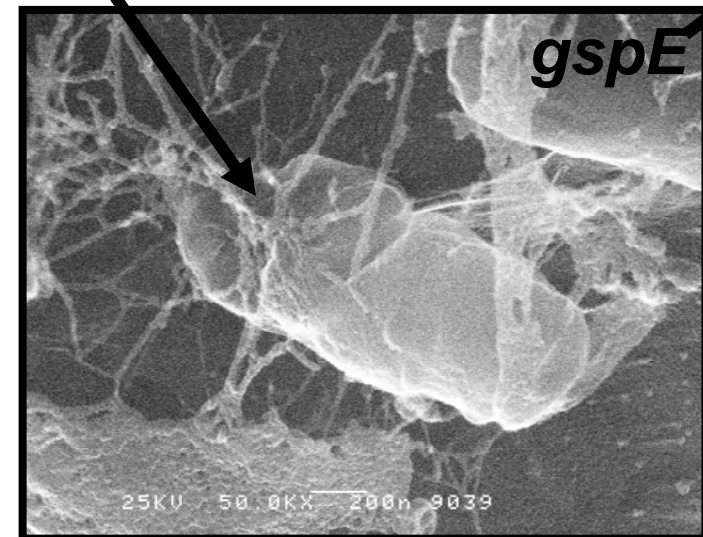
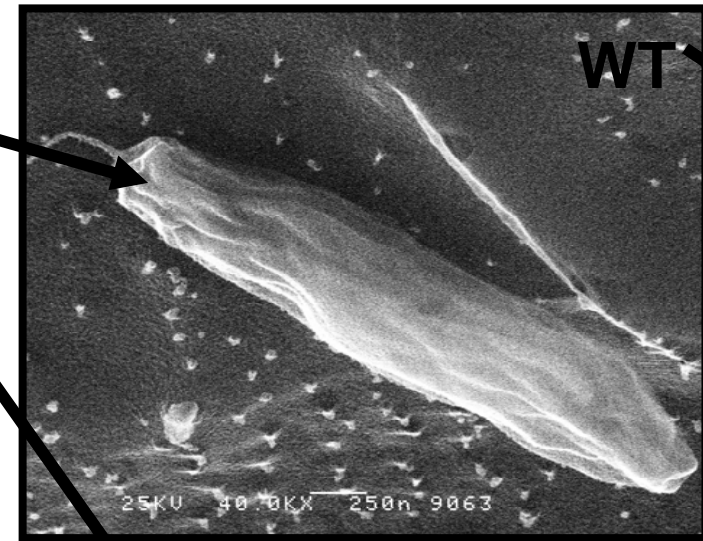
## Evolutionary history of MtrC of MR-1?



# *S. putrefaciens* 200 WT versus Type II secretion mutant *gspE* (in collaboration with Andy Neal at SREL)

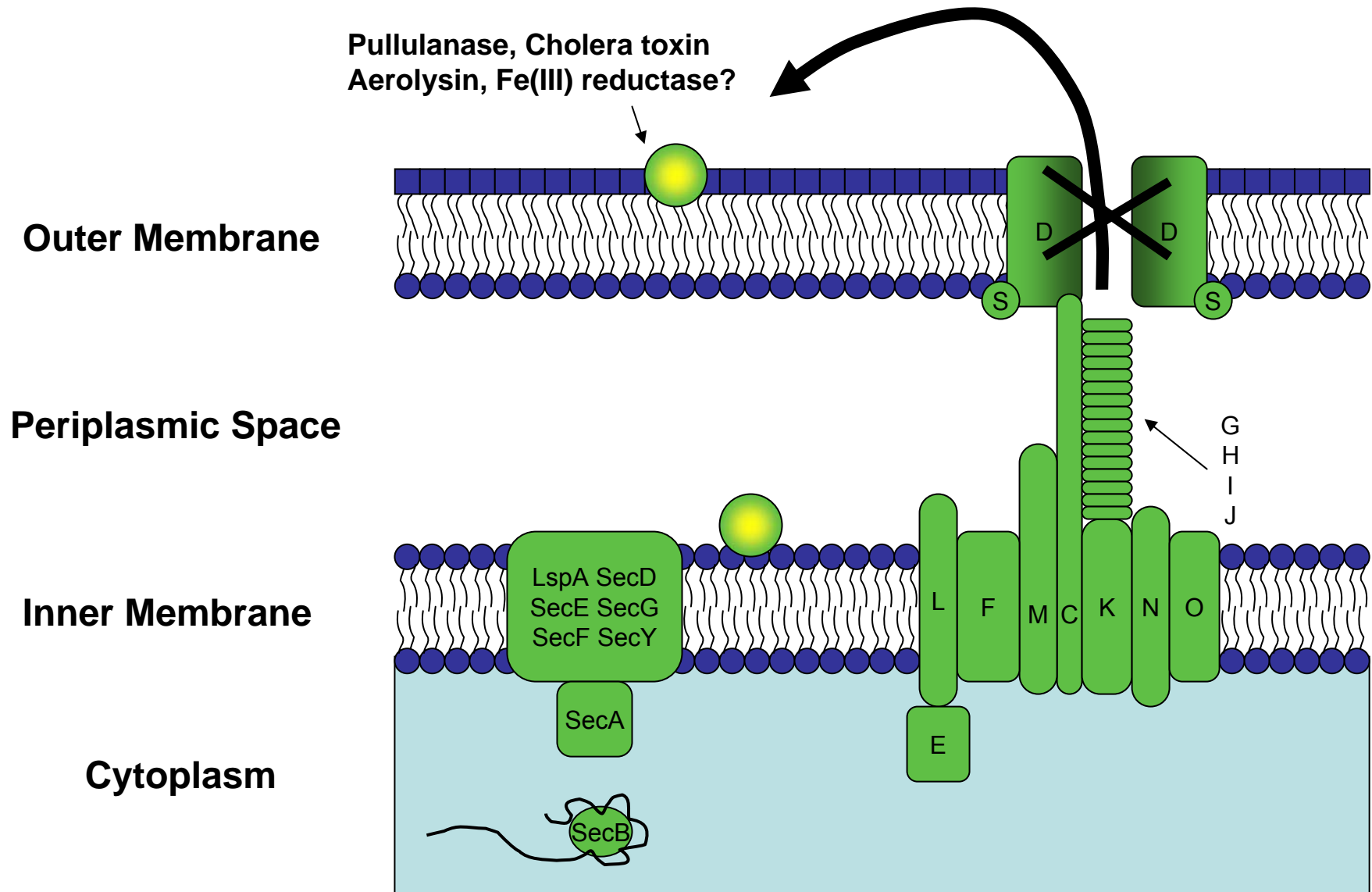
## Cryo HR-SEM

## Electrophoretic mobility

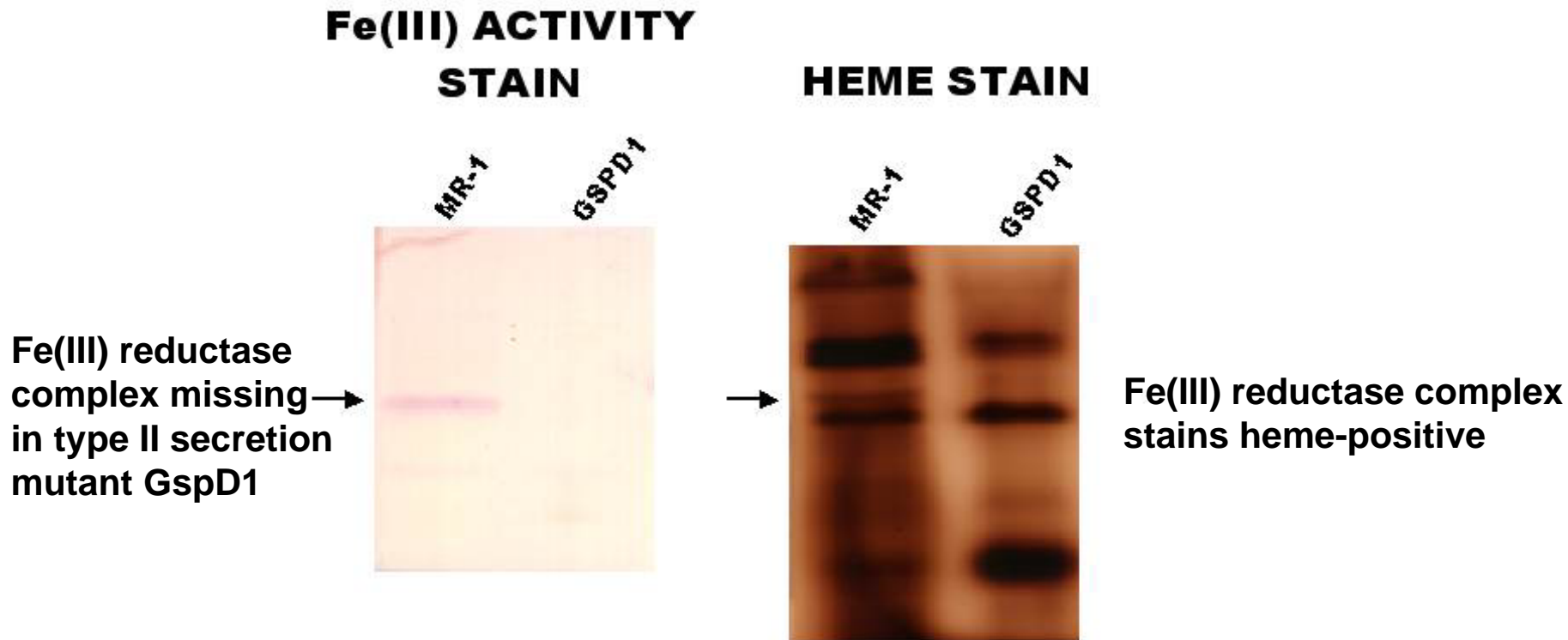


- Surface charge densities are similar
- GspE: thicker ion permeable layer (soft)
- Striking difference in surface features

# Site-targeted deletion of the MR-1 Type II secretin GspD

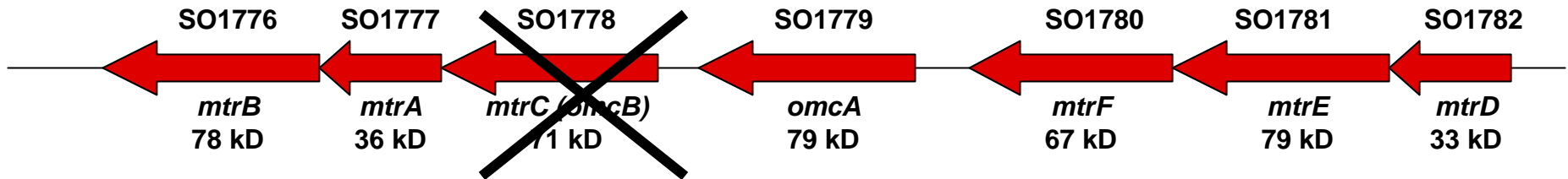


# Fe(III) reductase missing from peripheral proteins detached from outside face of *S. oneidensis* MR-1 outer membrane



MALDI-TOF MS analysis of heme-positive protein in Fe(III) reductase of *S. oneidensis* MR-1:  
MtrC

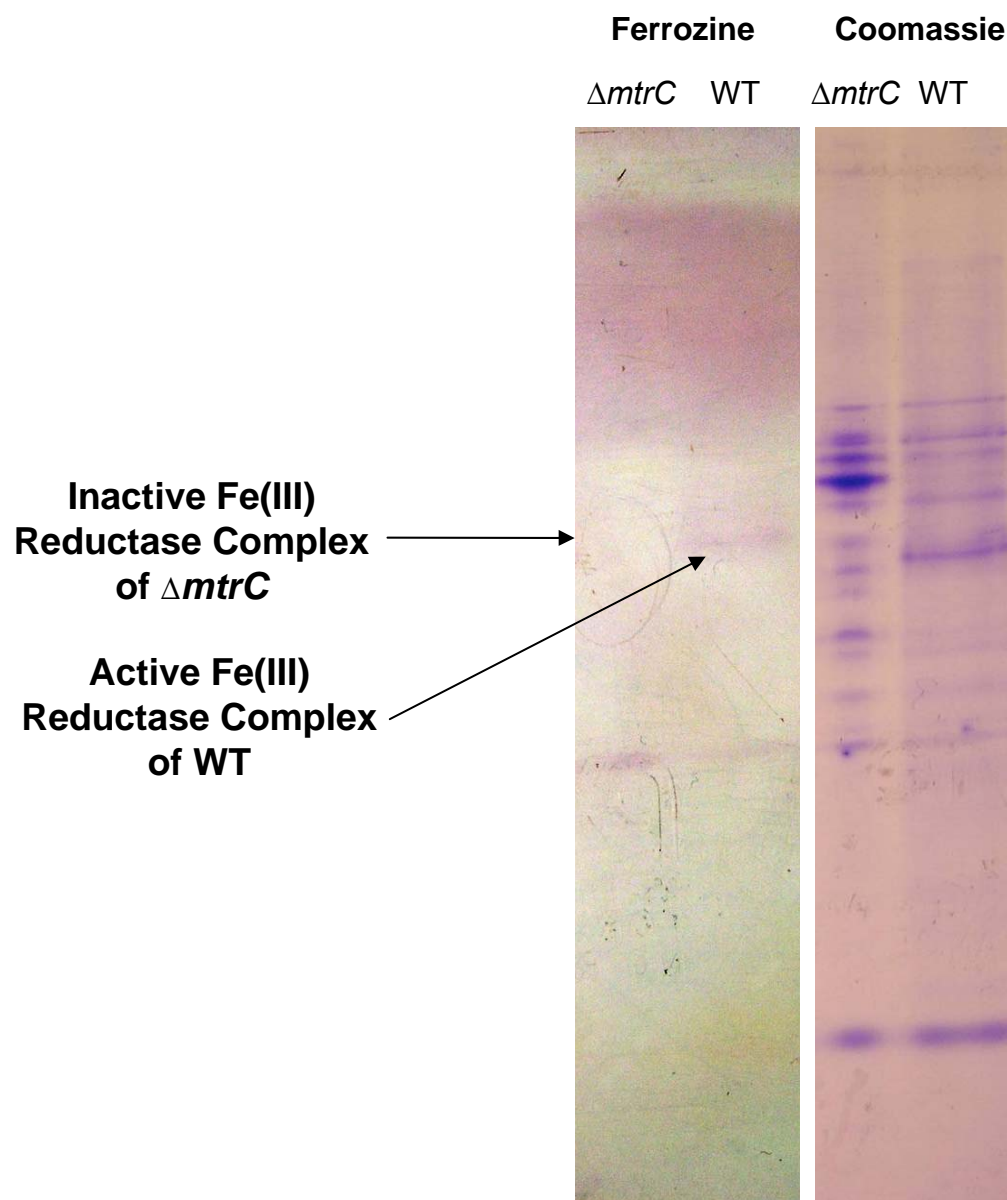
## Mtr region of *S. oneidensis* MR-1 genome



- MtrB:** putative outer membrane anchor (MtrE = second copy)  
**MtrA:** periplasmic decaheme c-type cytochrome (MtrD = second copy)  
**MtrC:** outer membrane decaheme c-type cytochrome (MtrF = second copy)  
**OmcaA:** outer membrane decaheme c-type cytochrome (no second copy)

**MtrC deletion mutant does not respire on solid Fe(III),  
and the activity of its Fe(III) reductase complex.....**

.....is severely impaired (Native PAGE Analysis of  $\Delta mtrC$ )



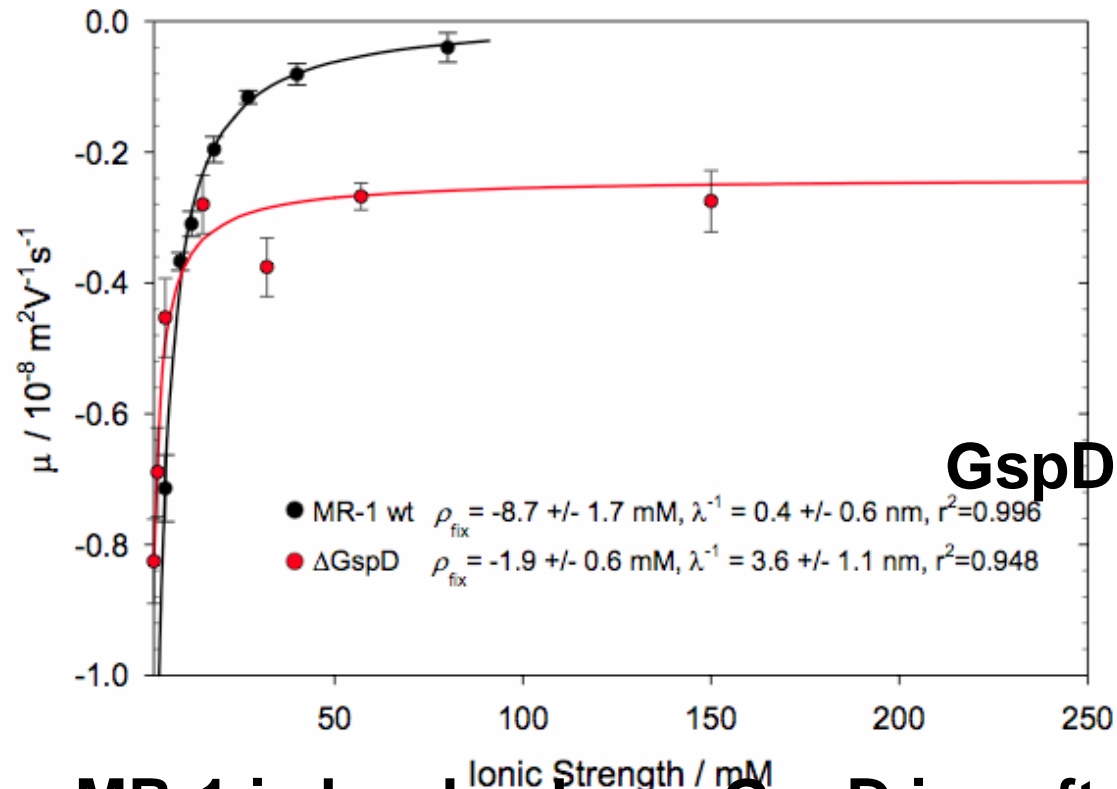
MtrC is required for Fe(III) reductase activity, but not for formation of Fe(III) reductase protein complex



# *S. oneidensis* MR-1 WT versus Type II secretion mutant *gspD*

MR-1

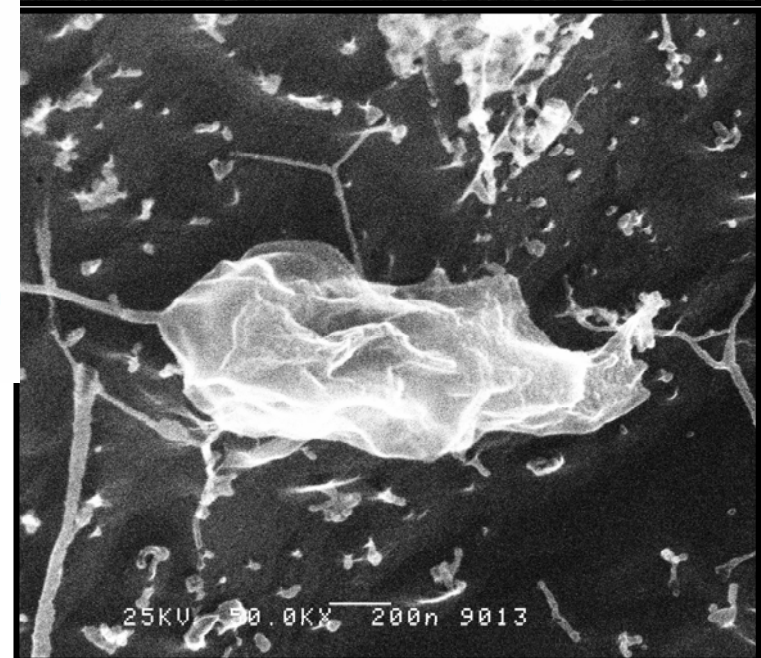
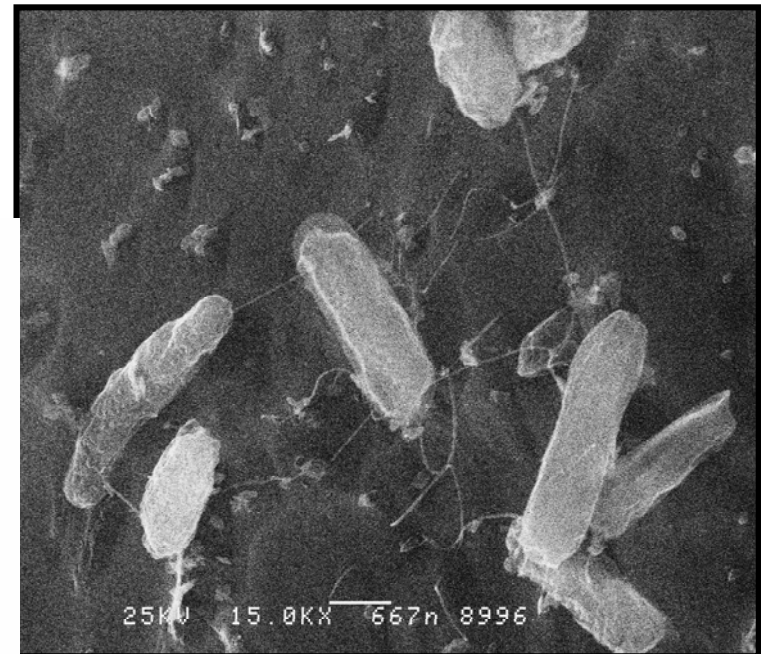
Electrophoretic mobility:



GspD

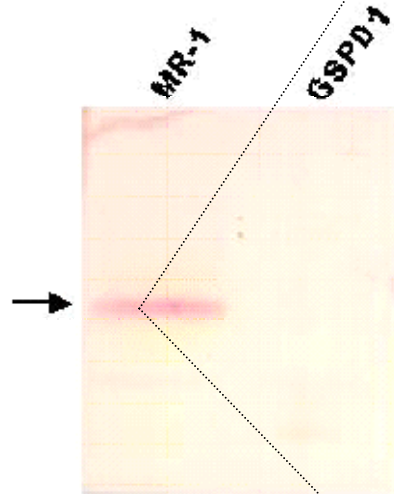
- MR-1 is hard sphere, GspD is soft
- MR-1 charge density is 5X higher
- GspD: ion permeable layer is 10X thicker and coated with EPS

Cryo HR-SEM:



# MALDI-TOF MS identification of polypeptides in Fe(III) reductase complex of *S. oneidensis* MR-1

## Fe(III) ACTIVITY STAIN



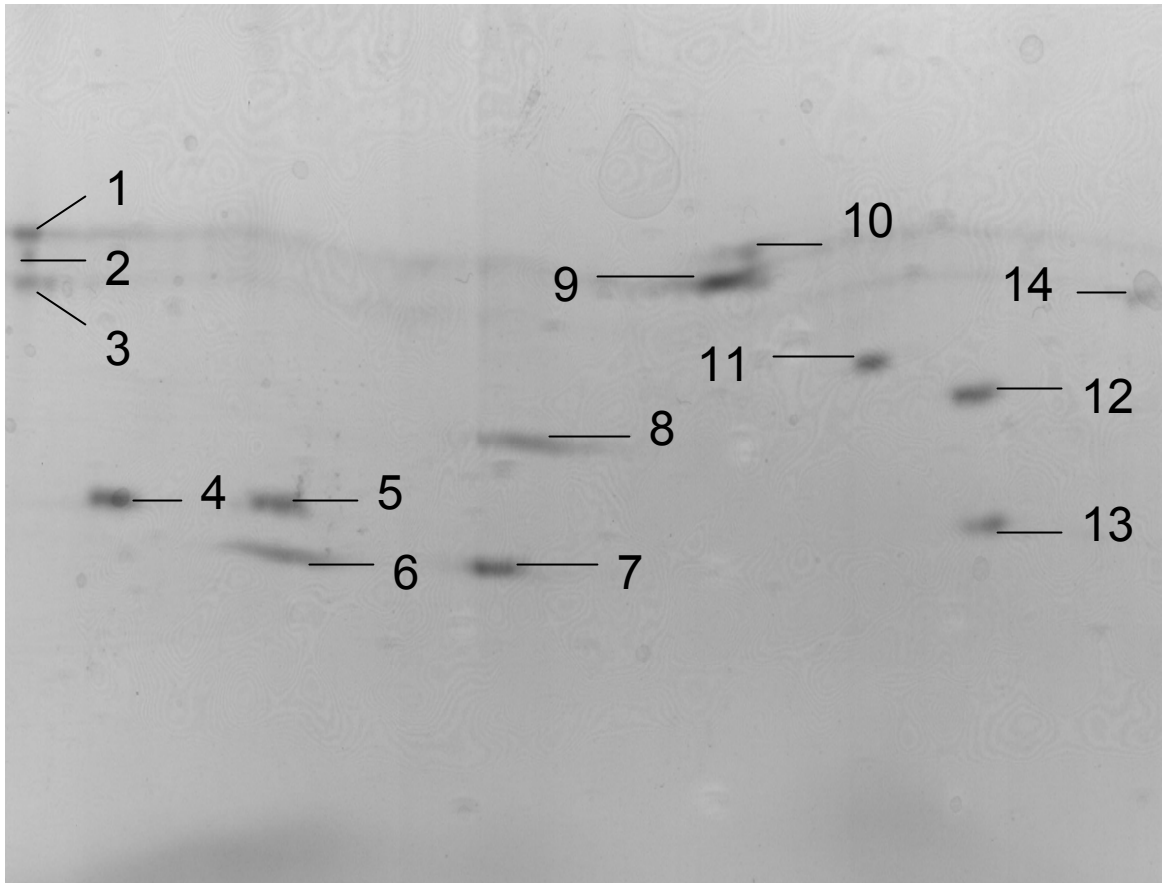
Autotransporter and one other protein

Autotransporter and two other proteins  
Autotransporter and two other proteins

Autotransporter and c-type cytochrome MtrC

Currently being studied

## 2-D SDS PAGE of Fe(III) Reductase Complex





# Secondary structure comparsion: autotransporters of *B. pertussis* and MR-1

\* BAD AVG GOOD  
 \*  
 BP3494 : 100  
 SO3800 : 100

BP3494 -----MYLDRFR-----QCPSSSLQIPRSAWRLHALAA  
 SO3800 MKTKLSLAISAALLTSAAVAGTTAQYNTTNQTDDKYA GLSVTKNDSNEOKQAVAWMVKLKAPS  
 Cons \* : : : \* . \* \* \* : : \* . :

BP3494 LALAGMAR-----LAPAAAQAPQPFVAGAPHAQDAGQE--GEFDHRDNTLIAVFDDGVGINLD  
 SO3800 LAQQSQLKGFNKQSVMSQIESSQTKVKNAITSMADADLKIVATTSKLVNSIIVEGNHKLQVLVSL  
 Cons \*\* . : . : : \* . \* . \* : \* \* . : . : \* : \* . : . : \*

BP3494 DDPDELGETAPPTLTKDIHISVEHKNPMSKPAIGVRV--SGAGRALTLAGSTIDATEGGT-----  
 SO3800 NNA--EVADILPIYDYKLDVAASA EYIKAKAVIDAGTASGKGQORVAVLDTGVVDYTHKALGGSGL  
 Cons : : \* : : \* . : : : : : : \* . \* . : : \* \* \* : : : : : \* \* . :

BP3494 -----FAVVERRGGTLELDGVTVAGGEGM---EPMTVSDAGSRLSVRGGVLCGEAPGVGLVRAA  
 SO3800 VADYKAAVAAKSEMPNWPQGVIGGWDFVNNDPNPIDVTTNHGTHVSHSVVGTAPDVELLVYS  
 Cons . \* . : : : . \* \* : : : \* . : : : : : : : \* \* \* . \* : :

BP3494 QGGQASIIDATLQSIILGPALIA DGGSISVAGGSIDMDMGPGFPFPFPPLPGAPLAAHPPLDRV  
 SO3800 VCNSGCSGIAQLNALEASMDPNGDGDISDRVDTVNMSLGGDFGDVEDGAVQVMINEMVOLGVN  
 Cons . . . . \* \* : : . . . . \* \* . : : : \* : \* . \* : : \* .

BP3494 AAVHAGQDGKV-----TLREVALRAHGPOATG--VYAYMPGSEITLQGG-----  
 SO3800 LVISAGNDGPTPFVVGGPSTTN SALS SVGAMTHPTTKVGKIEASIAGNTVTAVGAGFNKSNAYS  
 Cons . : \* : \* : . : . \* . : \* . : \* : \* : \* : \*

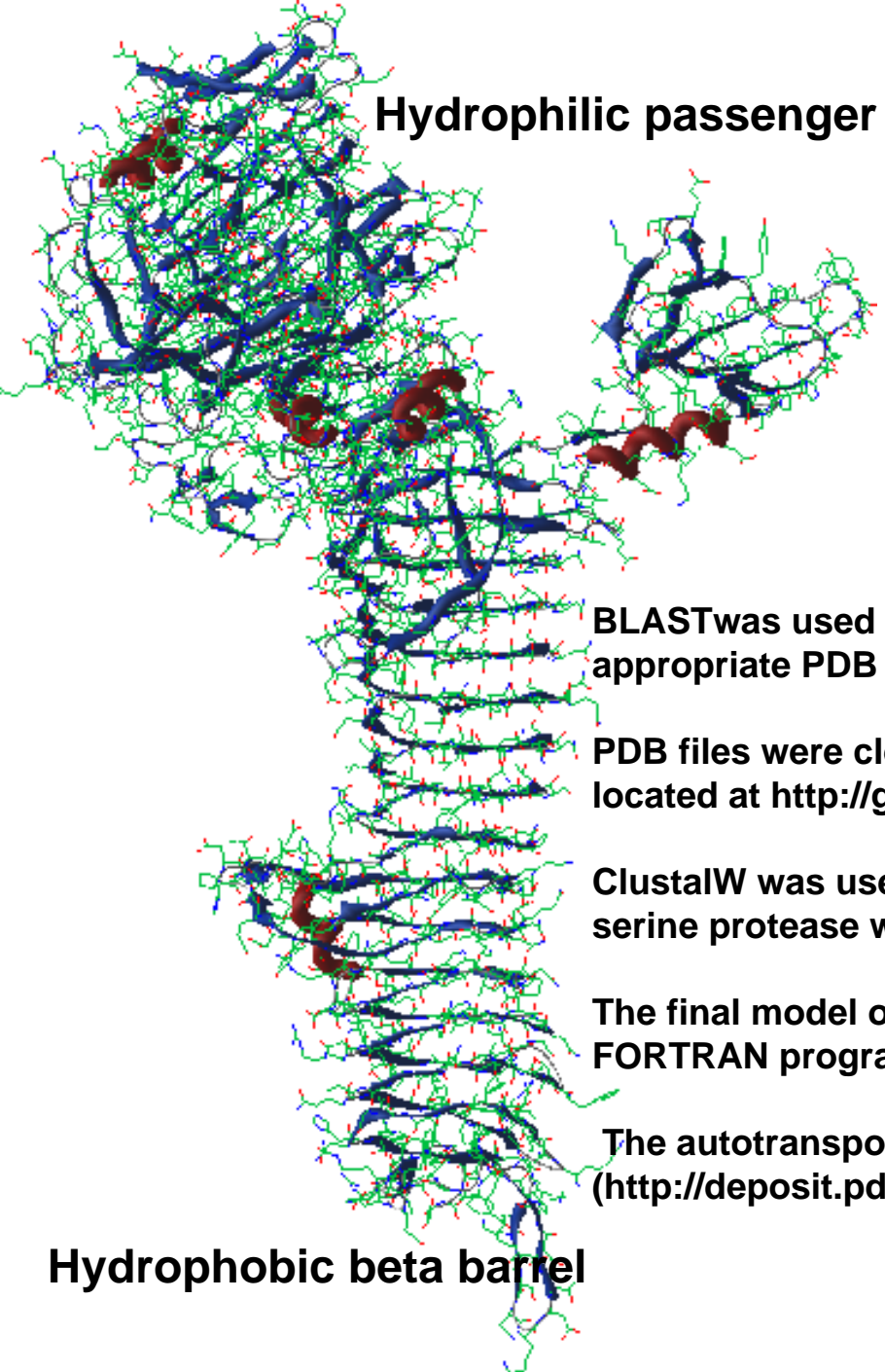
BP3494 -----TVSVQGDDGAGVVAGAGLLDALPPGGTVRLDGTT---VSTDGANT  
 SO3800 FTNTVAPIVYPAANKNGCTAYTEDLTGKTVLIDRGTCGFVVKVLNAQLKGASFVIVANNAANA  
 Cons \* . . : . \* \* : . \* . : : : \* . \* : : \* : : \* :

BP3494 DAVLVVRGDAARA EVVNTVLR T AKSLAAGVSAQHGG--RVTLRQTRIETAGA-----  
 SO3800 GAFVMGGTDDKITIPSVMVSKEDGDAIKTALASGDVAFSIASTELGTAGAIATFTSRGPSIGG  
 Cons \* . : : \* : : . : : . . \* . : \* . : : . \* : : \* :

BP3494 -----GAEGISVLGFEPOS GSGSPASVDMOGGSITTTGNRAAGI--ALTHGSA-----RL  
 SO3800 TLKPEITAPGTDILT AHPGLGEGLTPISGTSFSSPITAGAVSIIREALPHRNAFEVKATIMNA  
 Cons \* \* . : \* . \* \* : : . . \* . : : \* \* . : \* : \* . :

BP3494 EGVAVRAEGSGSSAAQLANGTLVVSAGSLASAQSGAISVTDTPFLKLMFGALASSTVSVRLTDG  
 SO3800 ANLDVTLEPKEINPDTTELAPISYIGSGLVDVEKAINLPVAAWNKDKTKQAALAFGLLALNKTT  
 Cons . : \* \* . . . : : \* : : : : \* . : \* \* . : : : \* .

BP3494 ATAQGGNGVFLQOQHSTIPVAVA-----LESGAL-----ARGDIVADGNKPLDAGISLSVASG  
 SO3800 ITKTVTVKNFSATEKTYTLKVDQRFQNDLDSGALS FALPASVTIPAGQTINFEDVTATIDPTKL  
 Cons \* \* . . \* . : \* : : \* \* : \* \* \* . \* \* . : \* . : : . :



## Homology modeling of MR-1 autotransporter

**BLAST** was used to search the protein databases for the most appropriate PDB structures to serve as templates for modeling.

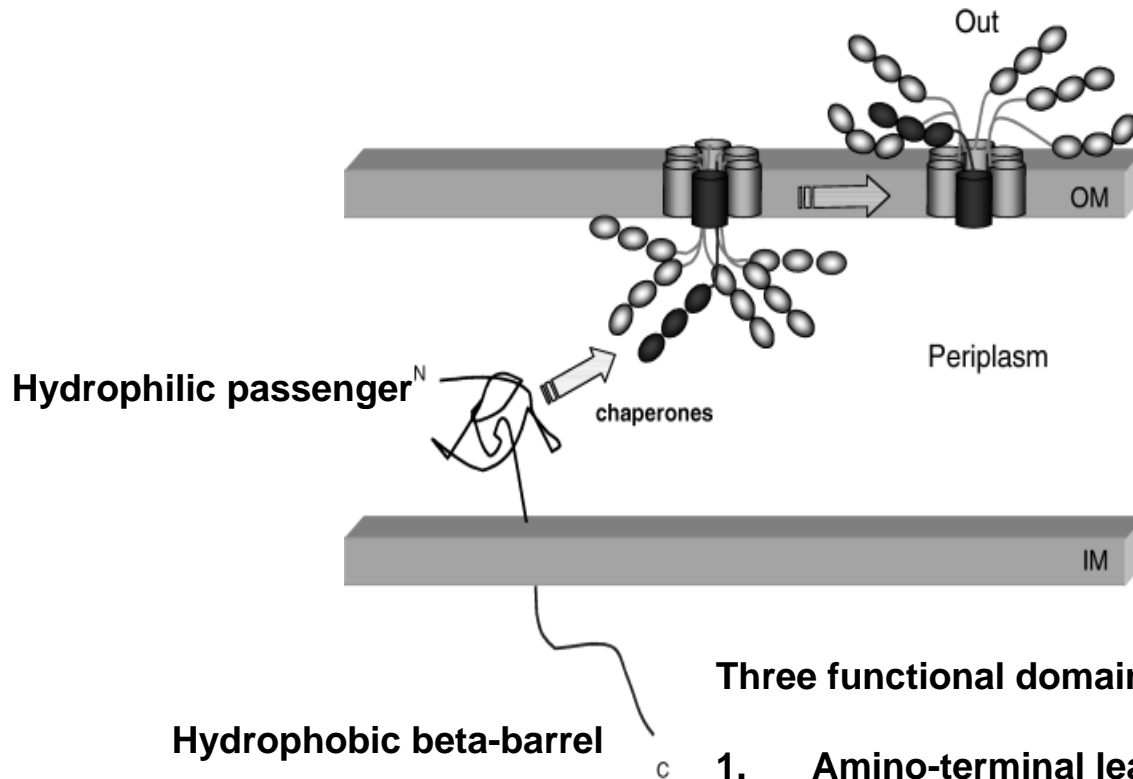
PDB files were cleaned-up using PDB Vacuum in the Modeler Toolbox, located at <http://genomics.biology.gatech.edu/motool>.

**ClustalW** was used to generate a multiple sequence alignment of the serine protease with templates that corresponded to conserved domains.

The final model of the autotransporter was generated with the FORTRAN program Modeller (<http://salib.org/modeller/modeller/html>).

The autotransporter homology model was validated with PROCHECK (<http://deposit.pdb.org/cgi-bin/validate/adit-session-driver>).

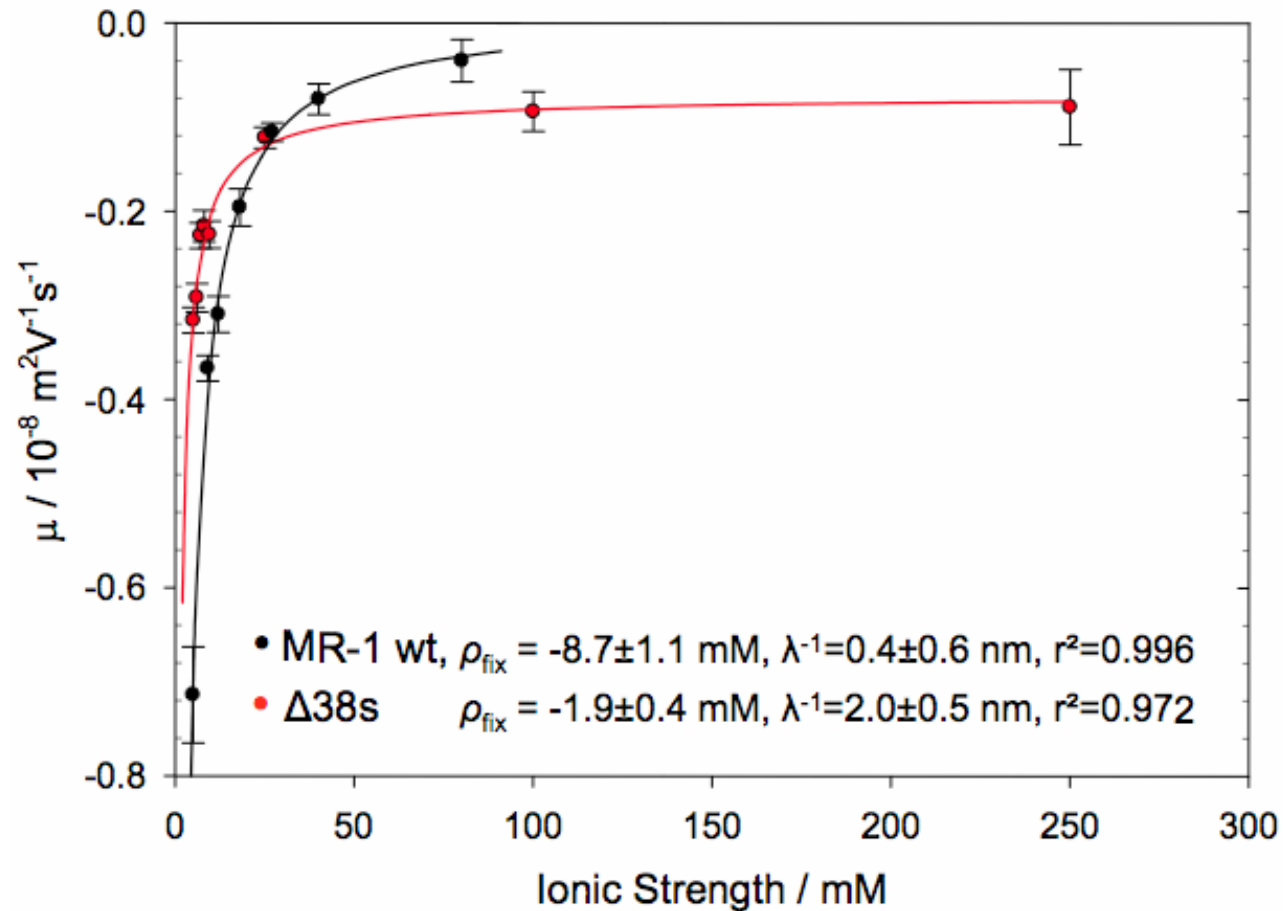
# Autotransporter (Type V Secretion)



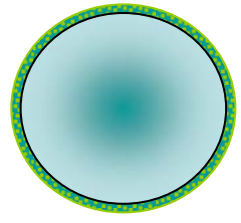
Three functional domains in autotransporters:

1. Amino-terminal leader sequence
2. Amino-terminal passenger domain
3. Carboxy-terminal domain that forms a hexameric beta-barrel pore to allow secretion of passenger domain

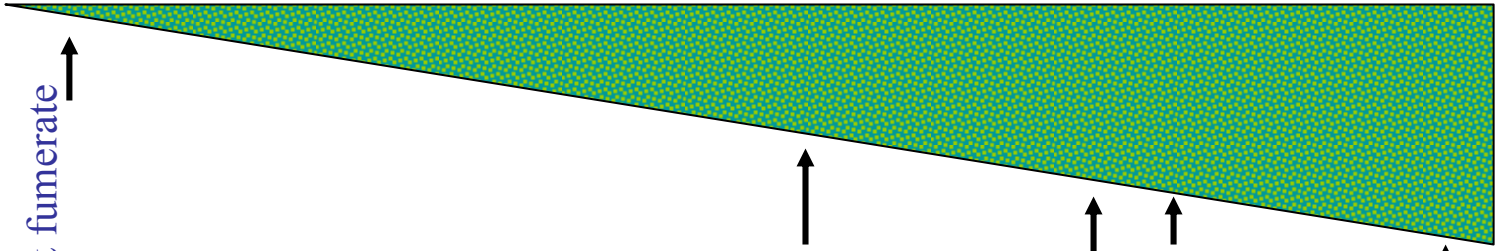
# Electrophoretic mobility of autotransporter mutant



- MR-1 is hard sphere, autotransporter mutant is 5X softer
- Autotransporter and GspD mutants are nearly identical in charge and softness
- Cryo HR-SEM not yet completed



*S. oneidensis* MR-1/nitrate & fumarate



*S. putrefaciens* 200R/  
nitrate & fumarate  
*S. putrefaciens* U14



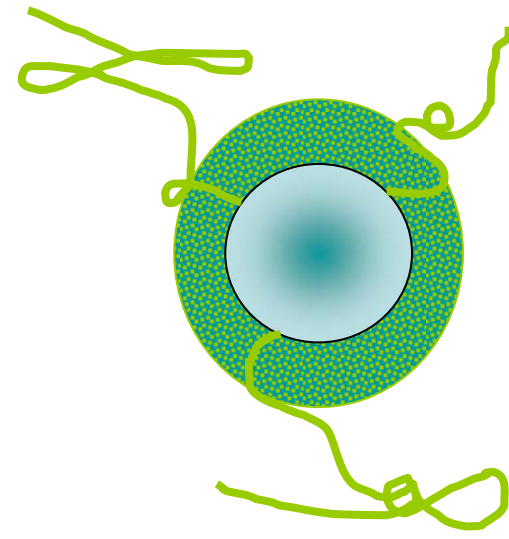
200R:: $\Delta$ gspE



*S. oneidensis* MR-1/ TMAO



MR-1:: $\Delta$ gspD  
*S. putrefaciens* 200R/ TMAO



# Working Model

